(Grant No. NS25656) and the Phoenix Memorial Laboratory of the University of Michigan for use of their facilities. We also thank Linder Markham and Pat Haines for typing the manuscript and Phil Sherman and Teresa Pisani for performing the tissue distribution studies.

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## Methylsulfamic Acid Esters. A New Chemical Class of Oral Antiarthritic Agents

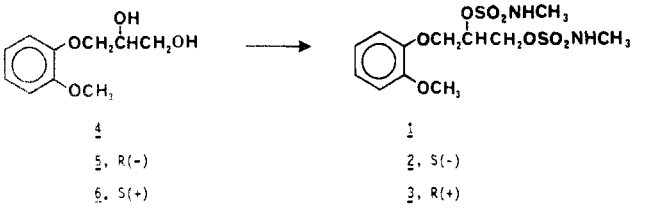
*Sir:*

Arthritis is a term used to describe a variety of disorders which manifest themselves in painful, inflamed joints.[1] Over the past 40 years therapies have been developed which treat various aspects of the disease states. These therapies include adrenal corticosteroids and the more potent synthetic derivatives; agents which interfere with arachidonic acid metabolism, the nonsteroidal antiinflammatory drugs (NSAIDs); the so-called disease-modifying antiarthritic drugs (DMARDs), the mechanism of action of which remains unclear; and the immunoregulatory agents, which affect the immune response. In this communication, we report on methylsulfamic acid 3-(2-methoxyphenoxy)-2-[[(methylamino)sulfonyl]oxy]propyl ester (**1**) and its optical isomers [**2**, *S*-(−); **3**, *R*-(+)], examples of the sulfamate ester pharmacophore, which have antiarthritic activity in the rat adjuvant-induced arthritis (AA) model[2] and which have a pharmacological profile that suggests that they exert their effects by a mechanism distinct from any agents previously reported.

The synthesis of **1** [mp 53.0–54.5 °C (isopropyl ether/1-propanol)] from commercially available **4** was readily accomplished in 82% yield as depicted in Scheme I. The optical isomers of **4**[3] were used to prepare **2** [mp 50.0–51.5 °C, $[\alpha]^{22}_D = -5.3°$ ($c$ = 1, $CH_3OH$)] and **3** [mp 50.5–52.0 °C, $[\alpha]^{22}_D = +5.3°$ ($c$ = 1, $CH_3OH$)]. Compound **4**, **5**, or **6** was dissolved in methylene chloride and treated over a 0.5-h period with simultaneous, dropwise additions of solutions of methylsulfamoyl chloride and diisopropylethylamine in methylene chloride. The reaction mixture was stirred at ambient temperature for an additional 2 h and then purified by column chromatography on silica gel to provide **1**, **2**, or **3**, respectively, as white solids.

Compounds **1**–**4** were tested in the adjuvant-induced arthritic (AA) rat model of chronic inflammation[2] by using a therapeutic dosing regimen[4] (dosing from day 18 through day 50 after adjuvant injection; Table I). Indomethacin at 3.16 mg/kg orally was used as a positive control. At

**Scheme I**[a]




**4** → **1**
**5**, R(−) → **2**, S(−)
**6**, S(+) → **3**, R(+)

[a] Reaction conditions: $CH_2Cl_2$, $CH_3NHSO_2Cl$, $C_2H_5N[CH(CH_3)_2]_2$, 25 °C, 2.5 h.

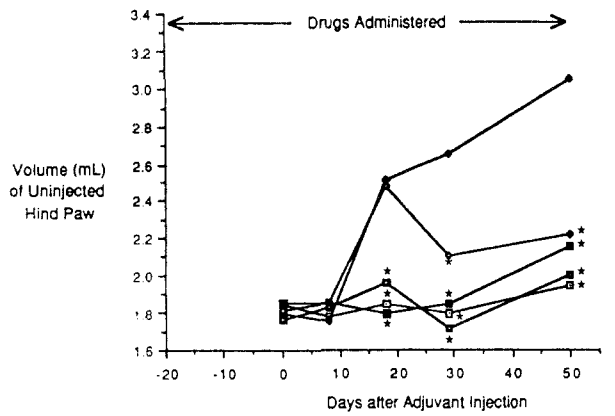



**Figure 1.** Effect of **1** (◇, 10 mg/kg), indomethacin (□, 3.16 mg/kg), and cyclosporin (■, 10 mg/kg) on the development of adjuvant arthrits in rats (◆, positive control; ⊡; negative control). The drugs were administered once daily to groups of rats beginning 18 days prior to the injection of the adjuvant. The volume of the uninjected hind limb was determined on days 0, 8, 18, 29, and 50 post adjuvant injection. An asterisk indicates that $p < 0.05$, compared to positive-control group, by Dunnett's $t$ test.

doses as low as 3.16 mg/kg, **1** showed significant activity against both edema (volume change of uninjected paw) and bone destruction (X-ray of uninjected joint). Interestingly, the *S*-(−)-isomer (**2**) was active at 10 mg/kg (the results for the 3.16 mg/kg dose of **2** were close to being statistically significant, but a larger number of animals will need to be tested to give statistical results comparable to those of the 3.16 mg/kg dose of **1**) while the *R*-(+)-isomer (**3**) was inactive at 31.6 mg/kg, the highest dose tested. Since **4** had no activity in the adjuvant arthritic rat assay at 100 mg/kg, the antiarthritic activity seen in **1** is not due to **4**, a potential metabolite.

When given daily at 10 mg/kg po, beginning 18 days prior to adjuvant injection, **1** did not inhibit generation of the arthritic state (unlike an immunomodulator or a steroid, but it did suppress the arthritic lesions once they were present (as in the therapeutic dosing regimen) (Figure 1). Histological analysis[5] revealed that **1** at 10 mg/kg po significantly suppressed the following parameters in adjuvant arthritic rats: osteolysis, chrondrolysis, synovitis, connective-tissue proliferation, and cellular inflammatory reaction. In addition, **1** at 10 mg/kg po had no significant effect on serum diagnostic values[6] (electrolytes, enzymes, etc.) of rats dosed from day 18 through day 50 after adjuvant injection or on weight changes in treated rats. These results (lack of effect on the development of arthritis

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(6) Roche Biomedical Laboratories, Richmond, Virginia.

0022-2623/90/1833-2068$02.50/0 

**Table I.** Oral Antiarthritic Activity in Rats with Adjuvant-Induced Arthritis Using a Therapeutic Dosing Regimen

| compd | dose, mg/kg | edema (mL ± SD) 11-day Δ[a] | edema (mL ± SD) 32-day Δ[b] | 32-day X-ray[c] (score ± SD) | weight change[d] | no. of rats |
|---|---|---|---|---|---|---|
| control | vehicle | 0.24 ± 0.25 | −0.07 ± 0.38 | 8.68 ± 1.22 | | 230 |
| indo[e] | 3.16 | −0.71 ± 0.23[f] | −0.77 ± 0.2[f] | 4.07 ± 0.72[f] | + | 228 |
| 1 | 10 | −0.42 ± 0.21[f] | −0.61 ± 0.27[f] | 5.77 ± 0.92[f] | NS | 210 |
| control | vehicle | 0.21 ± 0.23 | −0.01 ± 0.34 | 8.19 ± 1.79 | | 28 |
| indo | 3.16 | −0.74 ± 0.24[f] | −0.69 ± 0.27[f] | 3.80 ± 0.60[f] | + | 27 |
| 1 | 3.16 | −0.14 ± 0.34[f] | −0.38 ± 0.27[f] | 6.90 ± 1.08[f] | NS | 28 |
| 1 | 1.0 | 0.10 ± 0.24 | −0.23 ± 0.31[f] | 7.80 ± 1.34 | NS | 21 |
| control | vehicle | 0.10 ± 0.22 | −0.16 ± 0.16 | 7.54 ± 1.07 | | 7 |
| indo | 3.16 | −0.59 ± 0.16[f] | −0.66 ± 0.24[f] | 3.54 ± 0.39[f] | + | 7 |
| 1 | 10 | −0.39 ± 0.16[f] | −0.41 ± 0.24 | 5.82 ± 0.95[f] | NS | 7 |
| 2 | 10 | −0.33 ± 0.20[f] | −0.46 ± 0.18[f] | 4.96 ± 0.80[f] | NS | 7 |
| 2 | 3.16 | −0.11 ± 0.13 | −0.39 ± 0.16 | 6.64 ± 0.73 | NS | 7 |
| 3 | 31.6 | 0.16 ± 0.21 | −0.03 ± 0.15 | 7.86 ± 1.09 | NS | 7 |
| control | vehicle | 0.09 ± 0.22 | −0.27 ± 0.57 | 8.75 ± 1.07 | | 7 |
| indo | 3.16 | −0.70 ± 0.25[f] | −0.81 ± 0.24[f] | 4.04 ± 0.62[f] | + | 7 |
| 4 | 100 | 0.07 ± 0.16 | −0.40 ± 0.24 | 8.83 ± 0.68 | NS | 7 |

[a] Volume of uninjected rat paw on day 29 − volume of uninjected rat paw on day 18. [b] Volume of uninjected rat paw on day 50 − volume of uninjected rat paw on day 18. [c] X-rays were scored on an arbitrary (0 = normal to 10 = maximum joint destruction) scale in a blinded manner. [d] Weight change: + = significant increase in weight change from control; NS = not significantly different from weight change of control. [e] Indomethacin. [f] Significantly different from control at $p < 0.05$ by the Dunnett's $t$ test.

**Table II.** Effect of Multiple Oral Doses of 1 or of Indomethacin on Carrageenan-Induced Pleurisy in Rats[a]

| treatment | $n$ | dose, mg/kg, po | pleural volume, mL ± SD | % change from control |
|---|---|---|---|---|
| control | 6 | | 5.33 ± 0.87 | |
| indomethacin | 6 | 3.16 | 3.50 ± 0.91[b] | −34 |
| 1 | 6 | 4.00 | 5.20 ± 0.44 | −3 |
| 1 | 6 | 100.00 | 4.92 ± 0.69 | −8 |

[a] Twenty doses, one per day, administered prior to challenges. [b] $p < 0.05$, compared to the control group, by Dunnett's $t$ test.

**Table III.** Effect of Multiple Oral Doses[a] of 1, Cyclophosphamide, or of Cyclosporin on the Delayed-Type Hypersensitivity (DTH) Response to Methylated BSA (mBSA) in Sensitive Mice

| treatment | $n$ | dose, mg/kg, po | paw weight, mg ± SD | % change from positive control |
|---|---|---|---|---|
| positive control (sensitive mice) | 10 | | 183 ± 13.2 | |
| negative control (naive mice) | 10 | | 149 ± 9.6[b] | −19 |
| cyclophosphamide | 10 | 50.0 | 153 ± 9.0[b] | −16 |
| cyclosporin | 10 | 50.0 | 163 ± 16.0[b] | −11 |
| 1 | 10 | 31.6 | 179 ± 26.1 | −2 |

[a] Mice were sensitized to mBSA on day 0, dosed once with drugs on days 4, 5, 6, and 7 after sensitization, and challenged with mBSA in the left hind paw on day 7, and the paw weight was measured 24 h postchallenge. The negative control group was given Freund's complete adjuvant minus mBSA on day 0 and a challenge with mBSA on day 7. [b] $p < 0.05$, compared to positive control, by Dunnett's $t$ test.

or on weight changes) suggest that the effects of 1 are not nonspecific.

In acute models of inflammation such as the Evans blue carrageenan-induced pleural effusion assay[7] and the carrageenan-induced paw edema assay,[8] 1 was inactive at 100 mg/kg po. When given for 20 days prior to carrageenan challenge, 1 did not inhibit carrageenan pleurisy, even at doses of 100 mg/kg (Table II). Also, 1 showed no analgesic activity at 100 mg/kg po in the mouse acetylcholine writhing assay.[9] Platelet-activating factor (PAF) has been implicated in the inflammatory process,[1] but 1 at 100 mg/kg po did not block the formation of edema induced by PAF[10] in rat paws nor did it block the passive anaphylatic response in the foot pad of rats when given at 100 mg/kg. The results of these tests support our conclusion that the antiarthritic effects of 1 observed in the AA model are not due to nonspecific effects. At 31.6 mg/kg po, 1 did not affect a delayed-type hypersensitivity reaction in the mouse as did cyclophosphamide and cyclosporin (Table III).[11]

Biochemically, 1, 2, or 3 did not inhibit the cyclooxygenase enzyme from sheep seminal vesicles[12-14] ($IC_{50}$ > 500 μM) or enzymes of the 5-lipoxygenase pathway of human blood neutrophils[15-17] ($IC_{50}$ > 50 μM). When given

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(12) Lipid-depleted microsomes were prepared from sheep vesicular glands.[14] The lipid-depleted microsomal powder (20 mg) was solubilized in 1.0 mL of ice-cold 100 mM Tris-HCl buffer (pH 8.0) containing 1.0 mM phenol, 5.0 mM EDTA, 5.0 mM DDC, and 1.0% Tween-20 and was stored on ice until used. Cyclooxygenase activity was determined polarographically with a YSI Model 50 oxygen monitor.[15] Standard assays were conducted at 30 °C in 3.0 mL of reaction buffer, containing 100 mM Tris-HCl buffer (pH 8.0), 1.0 mM phenol, and 2.0 μM hematin. Detergent-solubilized microsomal powder (70 μg) was added to the reaction buffer and reactions were initiated by addition of 300 nmol of ammonium arachidonate. The instrument signal was digitized by a Beckman Instruments Co. digitmetry instrument coupler and acquired by a Hewlett-Packard Model 1000 minicomputer. Cyclooxygenase optimal velocities were calculated either from recorder tracings of oxygen concentration or from the digitized, differentiated instrument signal with the RSMTH-program of CALS (Computer Automated Laboratory System; Beckman Instruments Co.).

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to rats for 50 days at 10 mg/kg po, 1 did not alter the neutrophil arachidonic acid composition, nor did 1 alter the profile of lipid mediators generated by ionophore stimulation of human neutrophils (thus, 1 is not a phospholipase inhibitor).

Certain sulfamate esters have been reported[18] to possess carbonic anhydrase inhibiting activity. Carbonic anhydrase has been implicated in the mechanism of bone resorption,[19] which is an important component of rhematoid arthritis.[20] For this reason 1, 2, and 3 were assayed against carbonic anhydrase[21] but were found to be inactive ($IC_{50}$ > 16 μM). Also, the urine of rats receiving 10 mg/kg of 1 daily for 25 days did not inhibit carbonic anhydrase, indicating that no appreciable amount (<2% of dose) of metabolites which inhibited carbonic anhydrase[22] were present. Compound 1 was also devoid of any of the effects associated with carbonic anhydrase inhibitors such as diuretic[23] (10 mg/kg po, rats) or anticonvulsant activity (inactive at 100 mg/kg ip, mice, in the maximal electroshock assay[24]).

Summarily, 1 represents a new chemical class (the sulfamate esters) of antiarthritic agents which acts by a mechanism distinct from known therapeutic agents. This compound is active in the AA model in rats when given therapeutically or prophylactically. Unlike NSAIDs, steroids, or immunomodulators, 1 did not inhibit the generation of the arthritic state (day 18 edema, Figure 1). Compound 1 lacks nonspecific activity since it failed to inhibit carrageenan-induced, serotonin-induced, and PAF-induced edema or the passive-foot anaphylaxis response in rats, even at doses 10-fold in excess of those required for antiarthritic activity. This compound is active in a chronic animal model of inflammation but not active in acute models. Compound 1 has no effect on a DTH reaction, on arachidonic acid release, on the 5-lipoxygenase pathway, on cycloxygenase activity, or on PAF-mediated events. The fact that the *S*-(−)-isomer (2) is more potent than the *R*-(+)-isomer (3) in the AA rat assay suggests that a specific binding site, heretofore unreported to have an influence on the inflammatory process, may be mediating the diminution of the edema and bone changes.

Studies to discern the mechanism of action of this chemical class of compounds (over 400 sulfamate ester derivatives tested to date possess varying degrees of activity in the AA assay) are ongoing and will be reported in due course.

**Acknowledgment**. We thank Dr. Larry W. Jaques, Ashby F. Johnson, John B. Forehand, Joanne E. Anderson, and Malcolm D. Stone for determining the spectral and analytical data; Linda A. Anderson, Marianne H. Foxwell, Carol E. Gathright, Cynthia H. Radvany, and Laura L. Whitman for their technical assistance in conducting the biochemical and pharmacological assays; and Dr. David N. Johnson and Donna S. Souders for providing the anticonvulsant data.

(15) Human blood neutrophils were isolated by dextran sedimentation[17] and centrifugation on Ficoll/Hypaque.[18] Neutrophil cell suspensions in 1.0 mL of Dulbecco's medium ($1.7 \times 10^7$ cells/mL) were supplemented with 1 mM $CaCl_2$ and preincubated for 5 min at 37 °C with either ethanol or an ethanolic solution of 1, 2, or 3 (1–100 μM). Reactions were initiated by addition of an ethanolic solution containing a mixture of arachidonic acid and/or calcium ionophore (A23187). The final concentration of arachidonic acid and A23187 was 80 and 5 μM, respectively. At appropriate time periods, incubations were terminated by addition of 2 mL of acetone and assayed for 5-HETE, 12-HETE, 15-HETE, and $LTB_4$ formation. One nanomole of $PGB_2$ was added to the terminated reaction, and arachidonic acid metabolites were extracted on Baker-10 $C_{28}$ sample-preparation columns. One nanomole of 13-hydroxyoctadeca-9,11,15-trienoic acid was added to each sample before reconstituting them in 300 μL of 75% methanol in water. One hundred microliter aliquots were analyzed by HPLC (Ultrasphere 3u-ODS column). The column was eluted isocratically for 10 min with a solvent mixture composed of 30% solvent A [methanol/acetonitrile/water/phosphoric acid (25:25:50:0.02 v/v)] and 70% solvent B [methanol/acetonitrile/water/phosphoric acid (35:35:30:0.02 v/v)], followed by elution with a linear gradient that increased the component of solvent B from 70 to 90% over the next 15 min. From 25 to 45 min, the elution was continued isocratically with a mixture of 10% of solvent A and 90% of solvent B. Elution of hydroxy fatty acids, leukotriene B, and $PGB_2$ was monitored with a UV detector.

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*Received May 25, 1990*

## Nonsteroidal Antiinflammatory Drug Hydroxamic Acids. Dual Inhibitors of Both Cyclooxygenase and 5-Lipoxygenase

*Sir:*

The broad class of nonsteroidal antiinflammatory drugs (NSAIDs) are invaluable in the mainline treatment of rheumatoid arthritis and osteoarthritis.[1] The inhibition of arachidonate cyclooxygenase (CO) is a hallmark feature of virtually all marketed NSAIDs, and this property is believed to play an important role in their therapeutic efficacy.[2] However, the consequences of CO inhibition by NSAIDs result in certain mechanism-based side effects[3,4] including dyspepsia, gastrointestinal ulceration/bleeding, and nephrotoxicity, especially in the elderly population who use them most frequently.[1] The antisecretory and cytoprotective properties of prostaglandins have been well-studied,[5] and their role in cytoprotection

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